

Fractionation of Alginates by Precipitation with Calcium and Magnesium Ions

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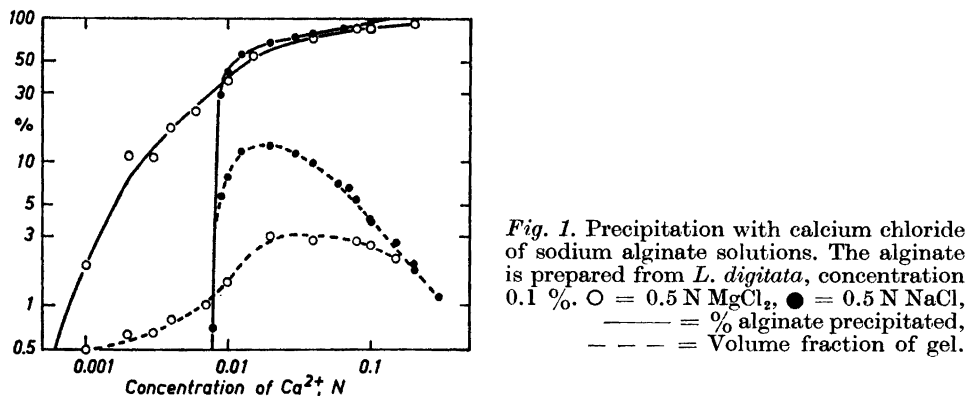
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Fractional precipitation of alginate with calcium ions in the presence of magnesium chloride leads to separation of the alginate into fractions with different uronic acid composition. A similar fractionation may also be achieved with magnesium chloride alone, in water or in the presence of small amounts of ethanol. In both cases the precipitates are enriched in guluronic acid-rich molecules. In the former case the fractionation is probably due to higher affinity for calcium ions of the guluronic-rich molecules in solutions of calcium and magnesium ions, while in the latter case a fractional salting-out of magnesium alginate in magnesium chloride solutions is responsible for the fractionation. These fractionation methods have considerable advantages compared to the methods earlier described.

Fractional precipitation has shown that ordinary preparations of sodium alginate are chemically heterogeneous. Material rich in mannuronic acid residues is preferentially salted out by potassium chloride,¹ whereas a guluronic acid-rich fraction is selectively precipitated by manganous ions.^{2,3} Divalent metals in general have a higher affinity for the more guluronic acid-rich alginates^{4,5} and manganous ions are particularly suitable as a precipitant for these materials because they give easily separable, flocculent precipitates. Other divalent metal ions, like those of calcium, give gels that occupy all or most of the volume of the solution,⁵ but the precipitate is less voluminous if a high concentration of sodium ions is also present; fractionations similar to those obtained with manganous ions are then possible.⁵ An improved method for fractionation, entailing the use of both calcium and magnesium ions, is now described. It gives a better separation than the methods described earlier,¹⁻³ and is simpler in use, due to the small volume of the precipitates obtained.

EXPERIMENTAL

Fractionation experiments were carried out on alginates prepared¹ from *Laminaria digitata* (Tarva 29/8), and from *L. hyperborea* stipes (Hustad 4/5). The two samples had, respectively, intrinsic viscosities of 18 and 15 dl/g, and mannuronic:guluronic acid



ratios⁶ of 1.6 and 0.5. Precipitation was carried out by mixing equal volumes of aqueous sodium alginate (0.2 or 0.5% w/v) with aqueous solutions of calcium or magnesium chloride. When calcium chloride was the precipitant, the second salt present (*i.e.* magnesium or sodium chloride) was dissolved in the alginate solution before mixing. All concentrations given in the figures refer to the final solution after mixing. The mixtures were stirred thoroughly for 1 min, allowed to stand for 30 min, and then centrifuged at $26\,000 \times g$ for 30 min. The precipitates were dissolved in aqueous ethylenediaminetetraacetic acid (2% w/v), and the amounts of material present in the resultant solution and in the original centrifugate were determined by the phenol-sulphuric acid method.⁷ The fractionations with potassium chloride and manganous sulphate, and the determination of the ionic selectivity coefficients were carried out as described earlier.^{1,3}

RESULTS AND DISCUSSION

Fig. 1 shows the percentage of the *L. digitata* alginate precipitated by various amounts of added calcium chloride, together with the volume of gel obtained after centrifugation, expressed as a percentage of the total volume of the mixture. Two different solvents, 0.5 N-magnesium chloride and 0.5 N sodium chloride, are compared, and it is observed that the gel volume is much the smaller with the former solvent. Fig. 2 compares the behaviour of the two alginates when precipitated by calcium chloride in the presence of different concentrations of magnesium chloride, and shows that the guluronic acid-rich alginate from *L. hyperborea* stipes has much the lower solubility. Magnesium alginate is considered to be soluble in water,^{8,9} but it is clear from Fig. 2 that guluronic acid-rich alginates have only a limited solubility in magnesium chloride solutions. This fact is further illustrated in Fig. 3, which compares the solubility of the two alginates in magnesium chloride solutions of various strengths; it is seen that, at magnesium chloride concentrations between 0.1 N and 1.0 N, much of the alginate from *L. hyperborea* is insoluble, whereas only small amounts of *L. digitata* alginate are precipitated. If precipitation of molecules less rich in guluronic acid is desired, this can be achieved without addition of calcium ions if the solubility of the magnesium alginate is diminished by addition of small amounts of ethanol; this is illustrated in Fig. 4 for *L. digitata* alginate.

Table 1 gives analytical figures for fractions obtained in these experiments, and figures for fractions obtained with potassium chloride and manganous

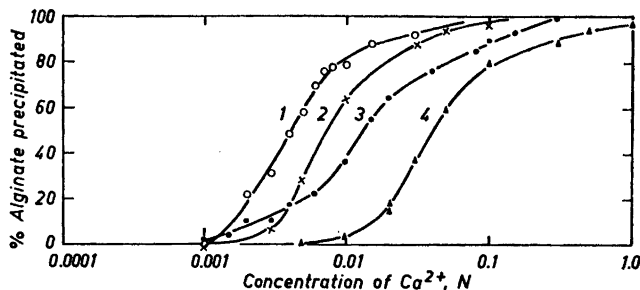


Fig. 2a. Alginate prepared from *L. digitata*, 0.1 %.

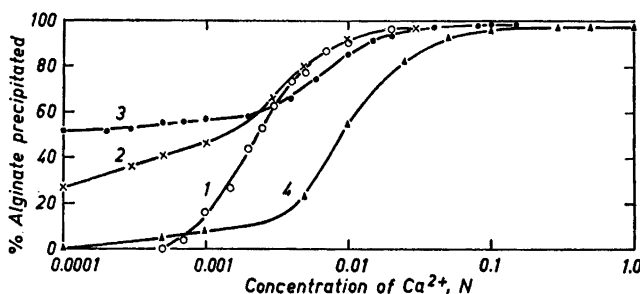


Fig. 2b. Alginate prepared from *L. hyperborea* stipes, 0.1 %.

Figs. 2a and 2b. Precipitation with calcium chloride of sodium alginate solutions containing different amounts of magnesium chloride. 1 = 0.08 N MgCl_2 , 2 = 0.125 N MgCl_2 , 3 = 0.5 N MgCl_2 , 4 = 1.2 N MgCl_2 .

ions are included for comparison. In some experiments, the soluble and insoluble fractions were further purified by refractionation under the same conditions; this is indicated in the column giving the number of fractionations.

The results show that efficient fractionation of alginates is possible by precipitation with calcium ions in the presence of magnesium chloride, and by precipitation with magnesium chloride, either alone or in the presence of ethanol. These methods give better separation than those described earlier,¹⁻³ and have the additional advantage that separation of the precipitates is much easier, and the volume of the gel very small.

Fractionation with calcium ions in the presence of magnesium chloride probably depends upon a difference in the relative affinity of calcium and magnesium ions for alginates containing different proportions of mannuronic and guluronic acid residues. This difference is illustrated in Table 2, which gives the selectivity coefficients for the calcium-magnesium ion-exchange reaction obtained with a number of different alginates.

The fractionation brought about by magnesium chloride alone, however, cannot be due to an ion-exchange reaction, since the conversion of sodium alginate into magnesium alginate was found to be more than 90 % at magnesium-ion concentrations lower than those leading to precipitation. The frac-

Table 1. Uronic acid composition of alginate fractions (M/G = ratio of D-mannuronic and L-guluronic acid residues).

Alginate sample	Fractionation method	Number of fractionations	% precipitated	Insoluble	M/G	Soluble
<i>L. digitata</i> , Tarva 29/8, M/G = 1.6	Potassium chloride	2	50	2.3	1.05	1.05
	Manganous chloride	2	50	1.03	2.0	2.0
	0.5 N NaCl + 0.012 N CaCl ₂	1	50	1.44	2.0	2.0
	0.5 N MgCl ₂ + 0.012 N CaCl ₂	1	50	1.06	2.6	2.6
	0.5 N MgCl ₂ + 0.012 N CaCl ₂	2		1.05	2.9	2.9
	0.3 N MgCl ₂	1	17	0.7	1.8	1.8
<i>L. hyperborea</i> stipes, Hustad 4/5, M/G = 0.5	0.15 N MgCl ₂ , 3 % ethanol	1	50	0.93	2.8	2.8
	0.08 N MgCl ₂ + 0.003 CaCl ₂	1	50	0.45	0.58	0.58
	0.4 N MgCl ₂	1	60	0.37	0.65	0.65

Table 2. Selectivity coefficients for the ion exchange reaction between calcium alginate and magnesium ions.

Alginate	M/G	$k = \frac{[Ca_g] \cdot [Mg_l]^*}{[Mg_g] \cdot [Ca_l]}$
<i>L. digitata</i> , Reine 10/2	2.10	3.8
<i>L. digitata</i> , Tarva 29/8	1.6	6.2
<i>L. hyperborea</i> stipes, Munkholmen 9/1	1.0	7.8
<i>L. hyperborea</i> stipes, Hustad 4/5	0.52	19
<i>L. hyperborea</i> stipes, outer cortex, Hustad 4/5	0.44	27

* The subscripts g and l refer to gel and liquid.

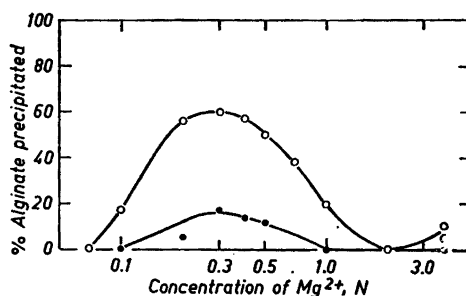


Fig. 3. Precipitation with magnesium chloride of sodium alginate solutions. O = Alginate prepared from *L. hyperborea* stipes, 0.25 %. ● = Alginate prepared from *L. digitata*, 0.25 %.

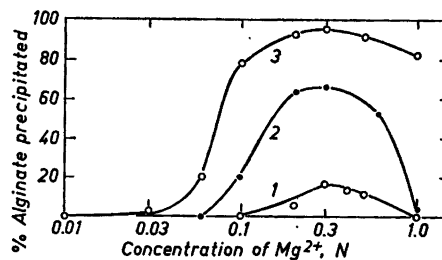


Fig. 4. Precipitation with magnesium chloride of alginate solutions containing different amounts of ethanol. The alginate is prepared from *L. digitata*, 0.25 %. 1 = Water, 2 = 3 % ethanol, 3 = 6 % ethanol.

tionation is therefore probably due to a fractional salting-out of magnesium alginate by magnesium chloride. At higher concentrations of magnesium chloride, the precipitate redissolves; a salting-in reaction takes place (Figs. 3 and 4). In this connection, it is of interest that, if magnesium sulphate is used instead of magnesium chloride, no precipitation of alginate takes place. Similar effects of the charge on the anion have been observed in the study of precipitation of charged polysaccharides by cation detergents.¹⁰ Further studies of the salting-in and salting-out of alginates and other polysaccharides are in progress in our laboratory.

REFERENCES

- Haug, A. *Acta Chem. Scand.* **13** (1959) 601.
- McDowell, R. H. *Chem. Ind. (London)* **1958** 1401.
- Haug, A. *Acta Chem. Scand.* **13** (1959) 1250.
- Haug, A. *Acta Chem. Scand.* **15** (1961) 1794.

5. Haug, A. and Smidsrød, O. *Acta Chem. Scand.* **19** (1965) 341.
6. Haug, A. and Larsen, B. *Acta Chem. Scand.* **16** (1962) 1908.
7. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. *Anal. Chem.* **28** (1956) 350.
8. Buchner, P., Cooper, R. E. and Wassermann, A. *J. Chem. Soc.* **1961** 3974.
9. McDowell, R. H. *Properties of Alginates*. Alginate Industries Ltd., London 1961.
10. Scott, J. E. *Methods Biochem. Anal.* **8** (1954) 145.

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